Phylogeny of *Willdenowia (Restionaceae)*: Implications for speciation mechanisms and evolution of regeneration strategies in the Fynbos.

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ABSTRACT

The phylogeny of *Willdenowia* is investigated using cladistic analysis, by studying culm anatomy, reproductive morphology and nut morphology. Two major clades are resolved, consisting of three and seven species respectively. Important characters being tepal shape, elaiosome and nut characteristics. Species groups within the genus are, in general, seperated by ecological factors, including altitudinal range, regeneration strategy (i.e. seeder/sprouter) and to a lesser extent phenology. This suggests that sympatric speciation, caused by steep ecological gradients present in the south-western Cape, may be an important process in the Fynbos. The *Willdenowia* phylogeny also shows that the ability to resprout after fire is ancestral, with sprouter-species giving rise to seeder-species in all cases. This suggests that sprouting is ancestral within the *Restionaceae*. This is discussed in relation to previous studies on regeneration strategies. This pattern also has implications for conservation; sprouter species possibly deserve a higher conservation status, as a result of their increased speciation potential. Because of their resiliance to the variable fire regime of the Fynbos, sprouter species are more likely to persist in the environment and give rise to new species, than are seeder species, which are more susceptible to local extinction.

1. INTRODUCTION

Elucidating the mechanisms behind the unusually high floral diversity of the Cape Flora remains as one of the most challenging biological problems of this unique area (Linder and Vlok, 1991). Two hypotheses have been advanced to explain the high species diversity of the Fynbos flora. Cowling (1987) invokes the effects of frequent, irregular fires which result in an absence of competitive exclusion as the dominant cause, while Linder (1985a) (Linder and Vlok, 1991), proposes that the steep ecological gradients present in the Cape region are the primary force behind increased speciation events.

One method of approaching this problem is to uncover processes operating at the generic level. Understanding processes at a generic level provides a "window" through which we can gain insight into processes occurring at a familial and landscape level. Through cladistic analysis, a putative phylogeny of a genus can be calculated. From a phylogeny, sister species can be isolated allowing the identification of mechanisms resulting in speciation events. This will, in turn, provide clues relating to the mechanisms behind the high species diversity of the Cape flora. The genus *Willdenowia* Thun. was considered a suitable genus for such a study, considering its size (i.e. eleven species) and its morphological variation.

To date the study of evolution and speciation within the *Restionaceae* has been limited to work on the genus *Rhodocoma* Nees (Linder and Vlok, 1991), a paper which provided valuable clues regarding speciation in the Cape Flora.

A second major aim of the study is to investigate the evolution of regeneration mechanisms within the genus *Willdenowia*. By assessing the distribution of seeders and sprouters on the phylogeny of *Willdenowia*, one can also gain insight into the processes behind the evolution of regeneration mechanisms. Pressing questions include the following: (i) Which state (i.e. seeder or sprouter) is ancestral ? (ii) Does the distribution of seeders and sprouters show any pattern, and if so, what are the implications of this pattern.

Willdenowia Thun. is a genus in the family *Restionaceae*, which includes eleven species. Thunberg, who originally erected the genus, included three species in his description (Linder, 1984). Pillans (1928), in his work on the African Restionaceae, expanded the genus to include twelve species, which was later increased to thirteen with the discovery and description of *Willdenowia stokoei* Pillans (Pillans, 1942). In his review of the African Restionaceae, Linder (1984) transferred *W.argentea, W.fistulosa and W.fimbriata* to *Ceratocyrum*. The description of *Willdenowia rugosa* Esterhuysen (Linder, 1985), brought the number of species in the genus to its current number of eleven.

The most important characteristic used to circumscribe *Willdenowia* is the form of the male inflorescences, which are thyrsoid (Pillans, 1928, Linder, 1984, 1985). Other important characteristics include linear male perianth segments, branching culms and the presence of an eliasome (the latter two characters are not consistent throughout the entire genus). The distribution range of the genus is centred in the south-western Cape, with individual taxa extending on a north-south axis (as far as Kamiesberg), or a west-east axis (as far as Knysna). Species are found across a wide altitudinal range, and are usually found to occupy occasionally damp habitats in the landscape.

2. MATERIALS and METHODS

All species of *Willdenowia*, excluding *Willdenowia affinis* Pillans, were included in the study. This species is known only from the type specimen collected on Table Mountain.

2.1 Reproductive structures.

Morphological data were collected from herbarium specimens. The youngest female inflorescence was selected for dissection. Material was reconstituted and softened in boiling soapy water, facilitating easier dissection, which was carried out under a compound microscope at 10X. To prevent dehydration while dissecting, material was placed in water in a petri dish. Dissected parts of the female inflorescence were flattened on a microslide which was coated with Prestik®, a pliable putty. This prevents the margins of bracts and tepals from becoming inrolled, which results in difficulty when inspecting dry material.

2.2 Stem anatomy.

Unblemished lengths of internode culm were cut and boiled in soapy water until soft. Specimens were sectioned using a sledge microtome, cutting at a thickness of between 20 μm and 30 μm. Sectioned material was stained in Safrinin-Alcian blue (Tolivia and Tolivia, 1987), dehydrated through an alcohol run, and then placed in xylene. Sections were mounted in Canada fixative on microslides, which were placed on a heating tray overnight. Microslides were dried in an oven for three days. Material was viewed under a light microscope between 10X and 200X.

2.3 Nuts.

Nuts were obtained from herbarium collections, and viewed under a dissecting microscope. Material was boiled to reconstitute eliasome tissue.

2.4 General.

Herbarium specimens were inspected for ecological information, including regeneration status and phenology. The ability to sprout after fire can easily be identified from herbarium material, by the charred remains of pre-fire culm growth combined with new post-fire vegetative growth.

Inferring phenology from herbarium material can be misleading. Male flowers are retained on the plant for differential periods after flowering, up to a few months in some cases. *Willdenowia* species also commonly retain stigmas until the nut is ripe, giving a false impression of recent flowering. An example of this includes *Willdenowia rugosa*; on closer inspection, apparently young female inflorescences reveal fully developed seeds. If one is aware of these difficulties, however, valuable phenological data can be obtained from herbarium specimens. Other information such as habitat type and geographical distribution were also obtained from herbarium material. A number of field trips were undertaken to acquire missing data relating to regeneration status and phenology.

The distribution of species are given in fig. 1 - fig. 5. Species generally occur across a wide geographical range, with individual ranges overlapping considerably.

2.5 Phylogeny.

Morphological and anatomical characters were scored onto a matrix (see Appendix 1 [Table

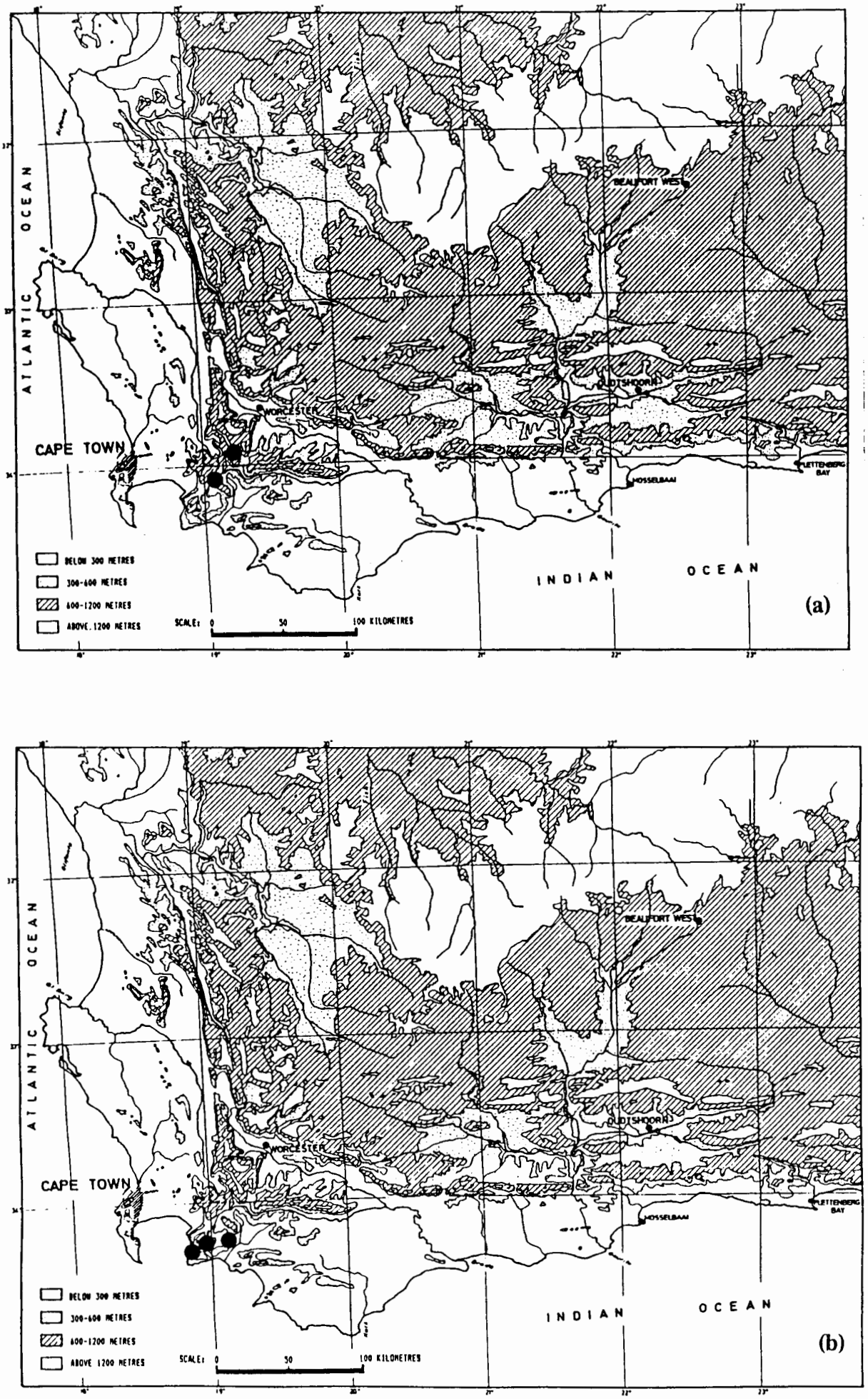


Fig. 1. Distribution of **(a)** *Willdenowia purpurea* and **(b)** *Willdenowia rugosa*.

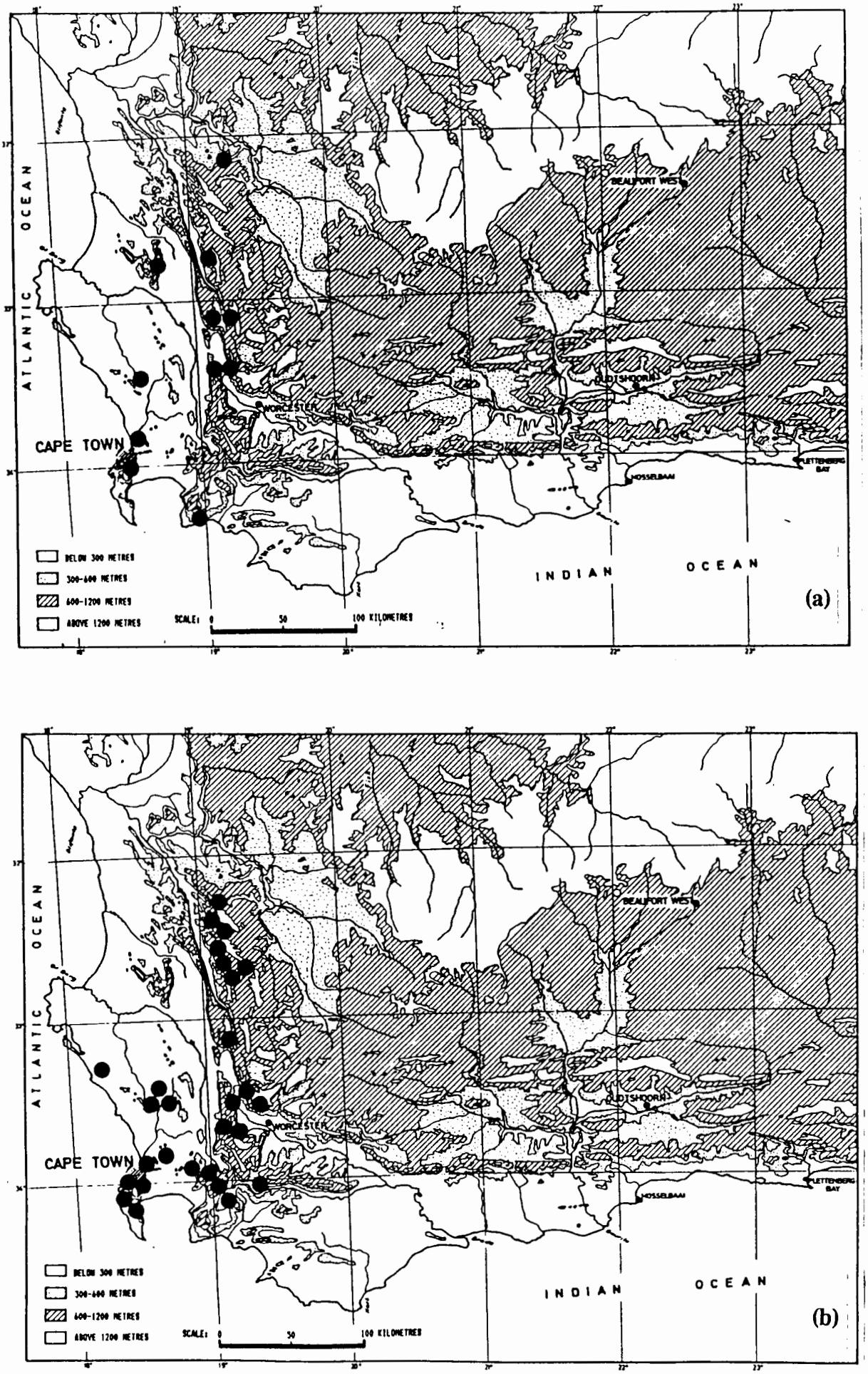
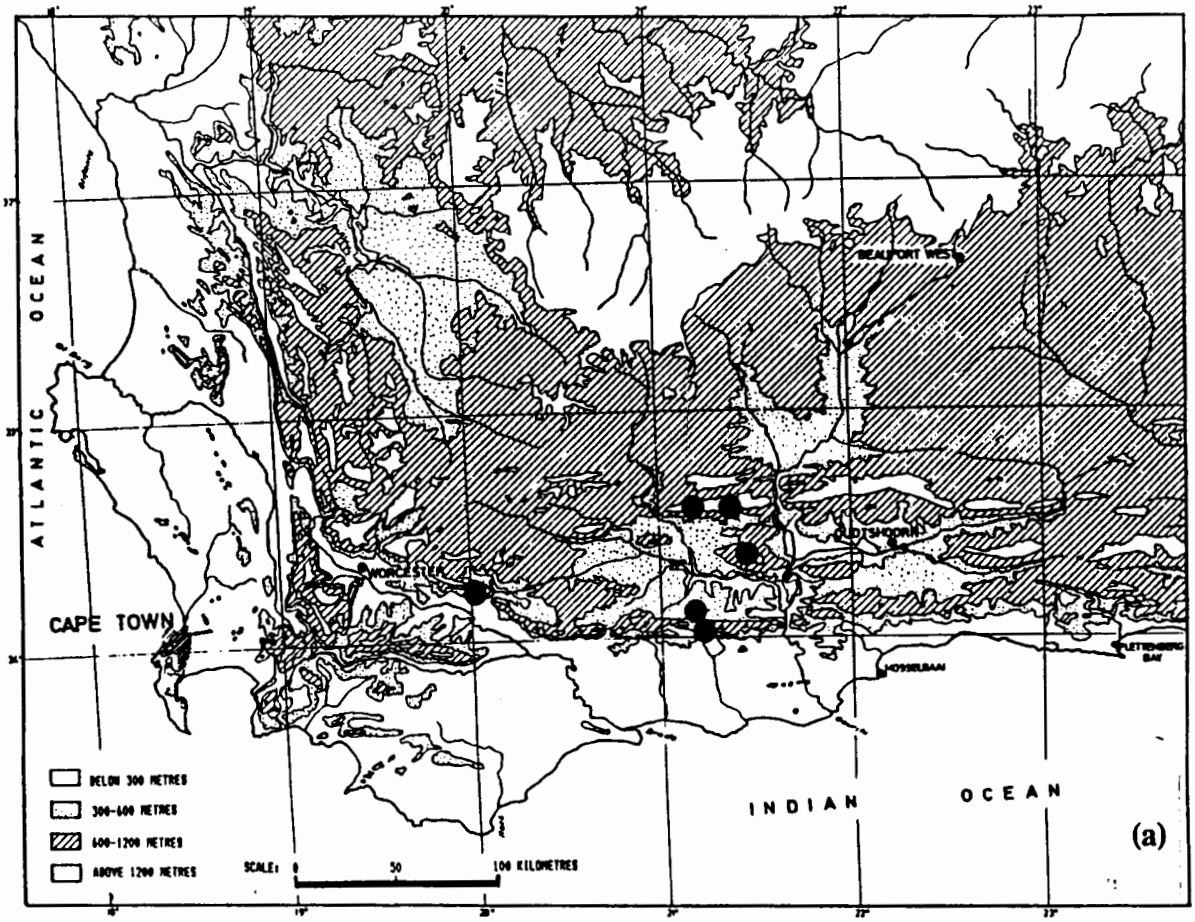


Fig. 2. Distribution of **(a)** *Willdenowia humilis* and **(b)** *Willdenowia sulcata*.



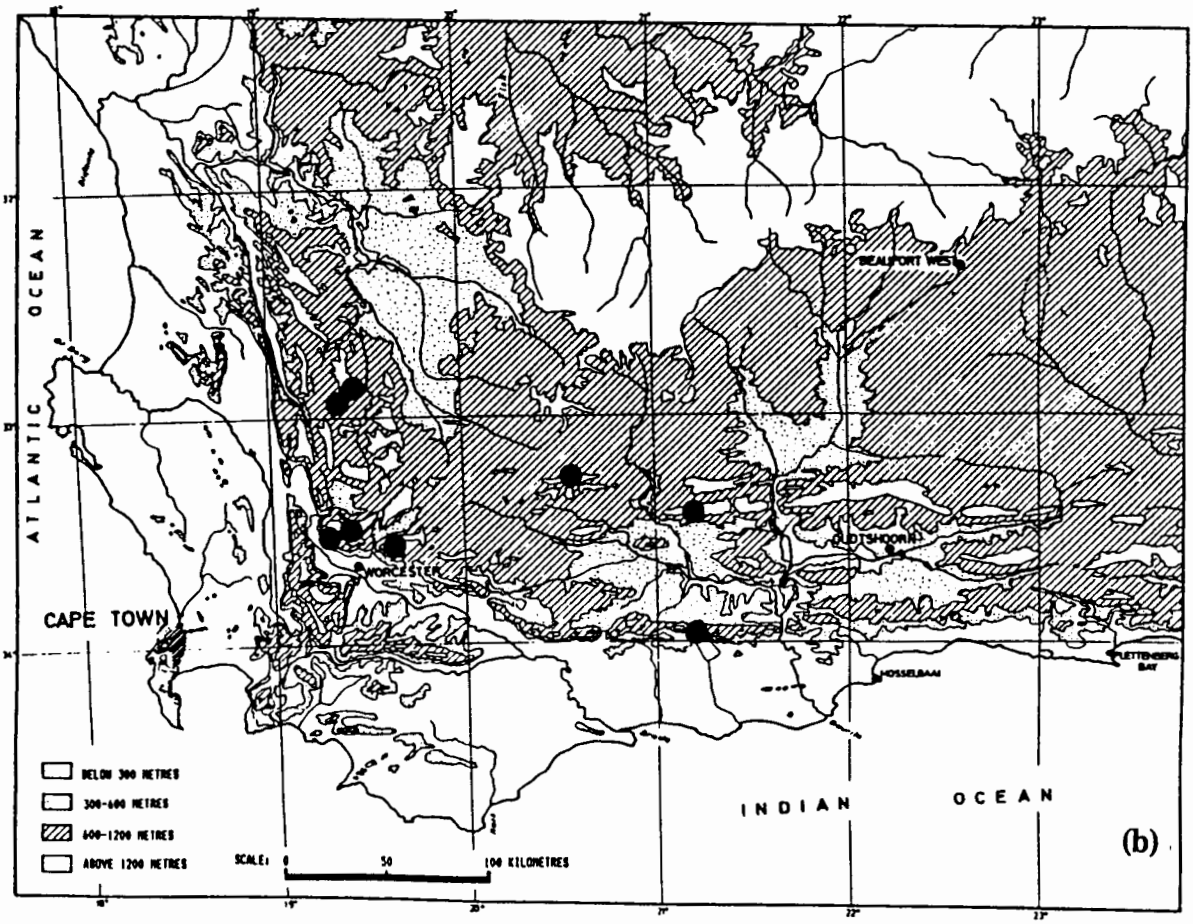


Fig. 3. Distribution of (a) *Willdenowia bolusii* and (b) *Willdenowia stokoei*

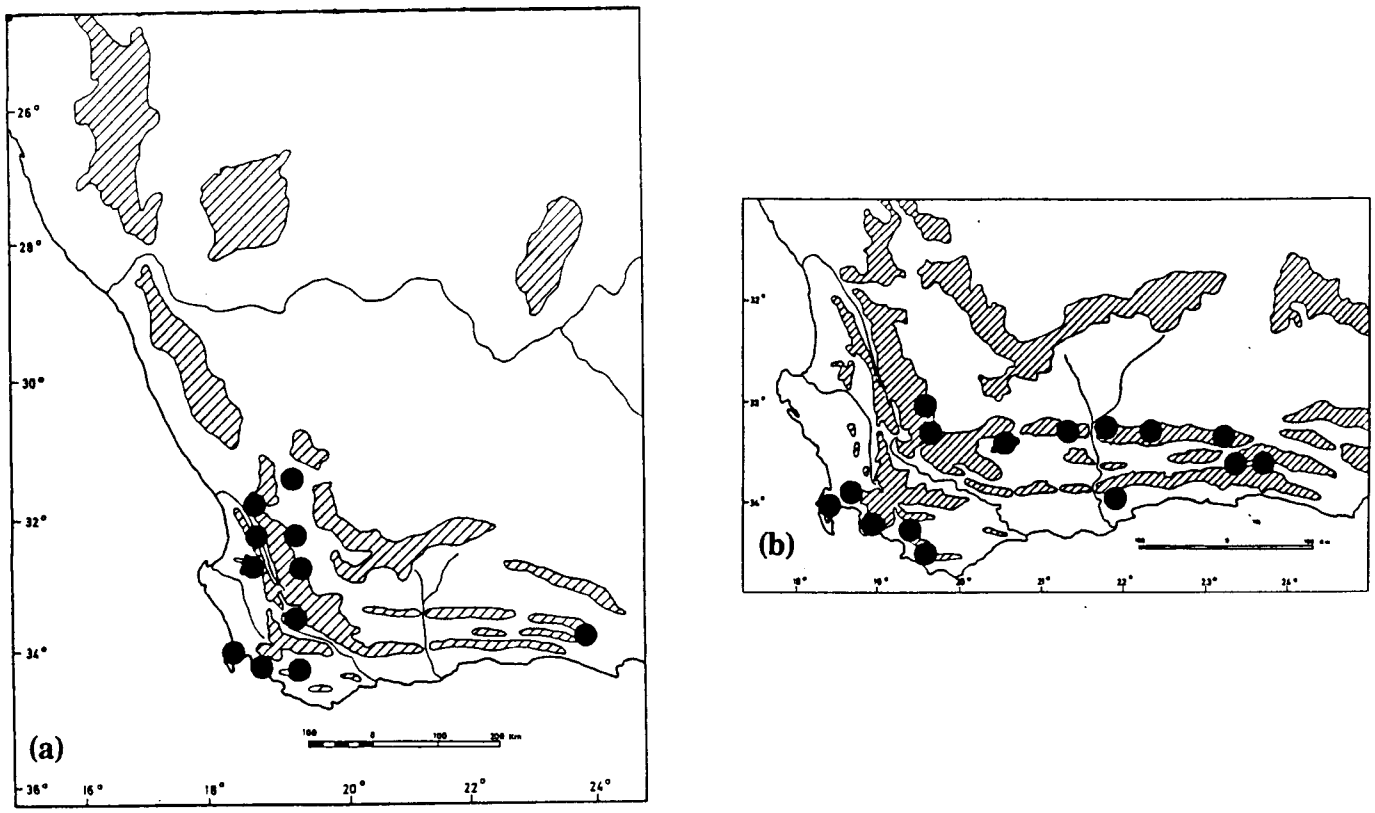


Fig. 4. Distribution of **(a)** *Willdenowia glomerata* and **(b)** *Willdenowia teres*.

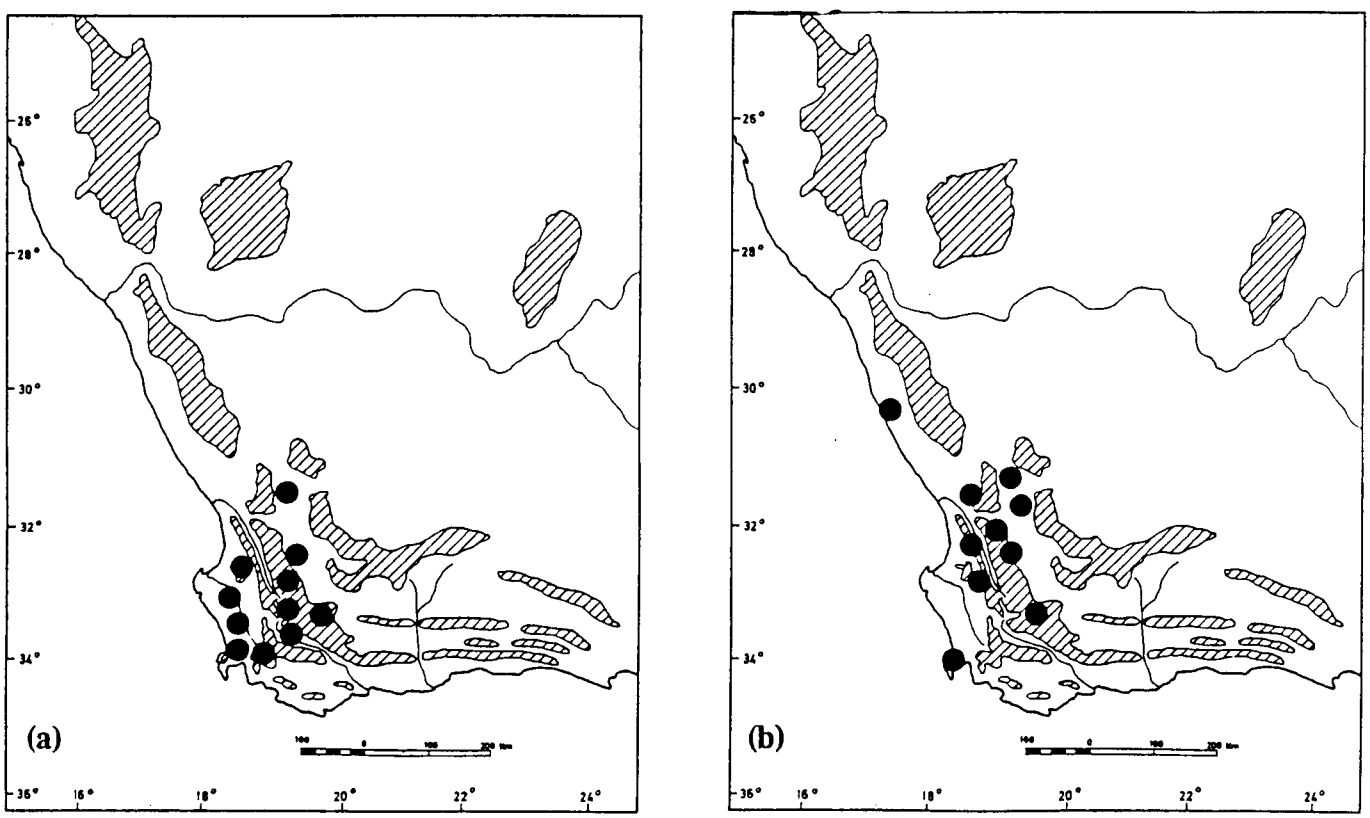


Fig. 5. Distribution of **(a)** *Willdenowia arescens* and **(b)** *Willdenowia incurvata*.

1] for a list and description of characters used). Character polarity was assessed using outgroup comparison (Maddison *et al.*, 1984, as cited by Linder and Vlok, 1991). According to the phylogeny of the African Restionaceae produced by Linder (1984), the sister-group (and therefore outgroup) to *Willdenowia* is *Hypodiscus* Nees. *Hypodiscus aristatus* (Thunb.) Krauss was chosen from this genus to be the outgroup in this study. Cladistic analysis was carried out using Hennig86 (Farris, 1988). The character set was analyzed using the ie routine, which calculates minimal length trees by implicit enumeration (Platnick, 1989). A Nelsen consensus tree was calculated using the nelsen routine (Page, 1989). Boot strap analysis was used to test condfidence limits for different nodes of the cladogram (Sanderson, 1989), while Random cladistics (written by M. Ciddall) calculated the statistical significance of the cladogram.

3. RESULTS

3.1 Phylogeny.

Cladistic analysis located five minimal length trees of 64 steps, with ci of 53 and ri of 55. Successive weighting of these five trees produced a single tree with ci of 83 and ri of 86 (fig. 6). This single tree was found to contain hierachical information at a significance level of <0.001 ($p=0.009901$). Five nodes collapsed when a Nelsen consensus tree was calculated (fig. 7). Boot strap analysis, which measures the stability of nodes of the cladogram, did not produce good results (fig. 7). All nodes within the second major clade had bootstrap values of below 50%.

Ecological features were mapped onto the cladogram (fig. 8), as were changes in tepal shape (fig. 9 and fig. 10).

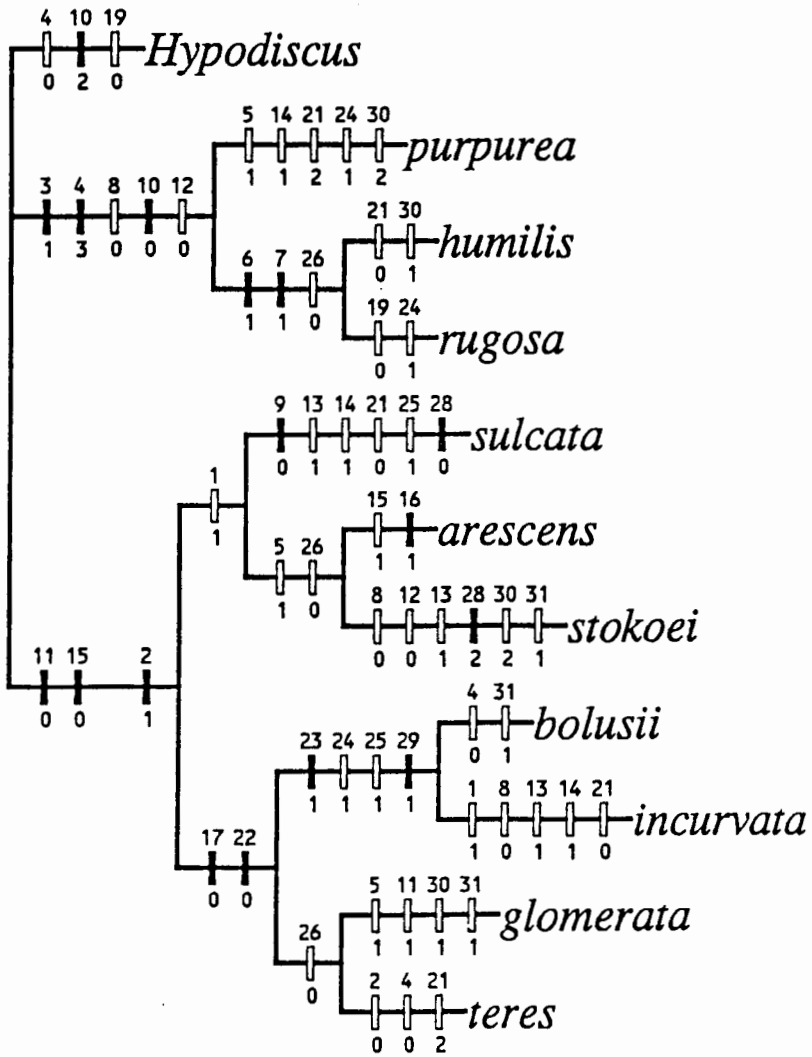


Fig. 6. Cladogram for the species of *Willdenowia*. The characters are according to Table 1. Synapomorphies are represented by solid bars, reversals are represented by hollow bars.

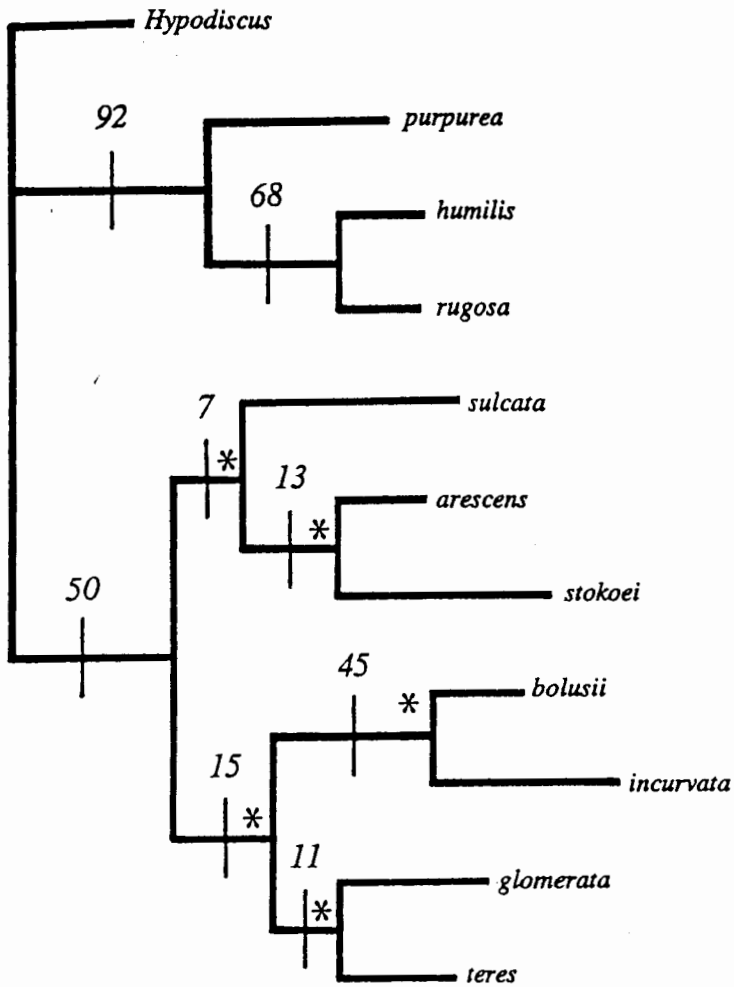


Fig. 7. Results of boot-strap analysis, testing the confidence limits for nodes of the *Willdenowia* cladogram. Asterisks represent those clades which collapsed when a Nelsen consenus tree was calculated.

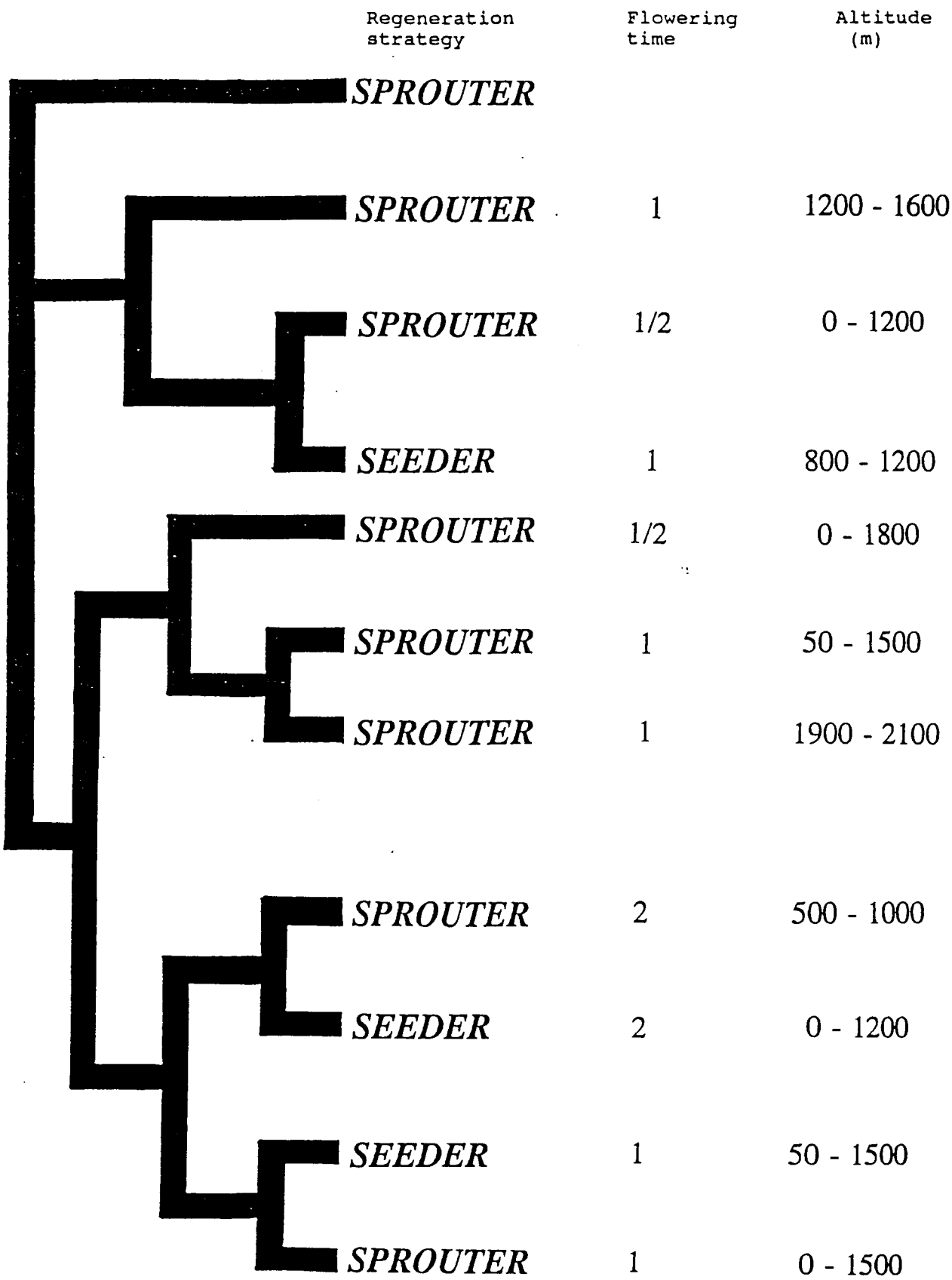


Fig. 8. Figure showing an overlay of regeneration status, flowering time and altitude range on the *Willdenowia* cladogram.
[For flowering time, 1=September to November, 2=May to June]

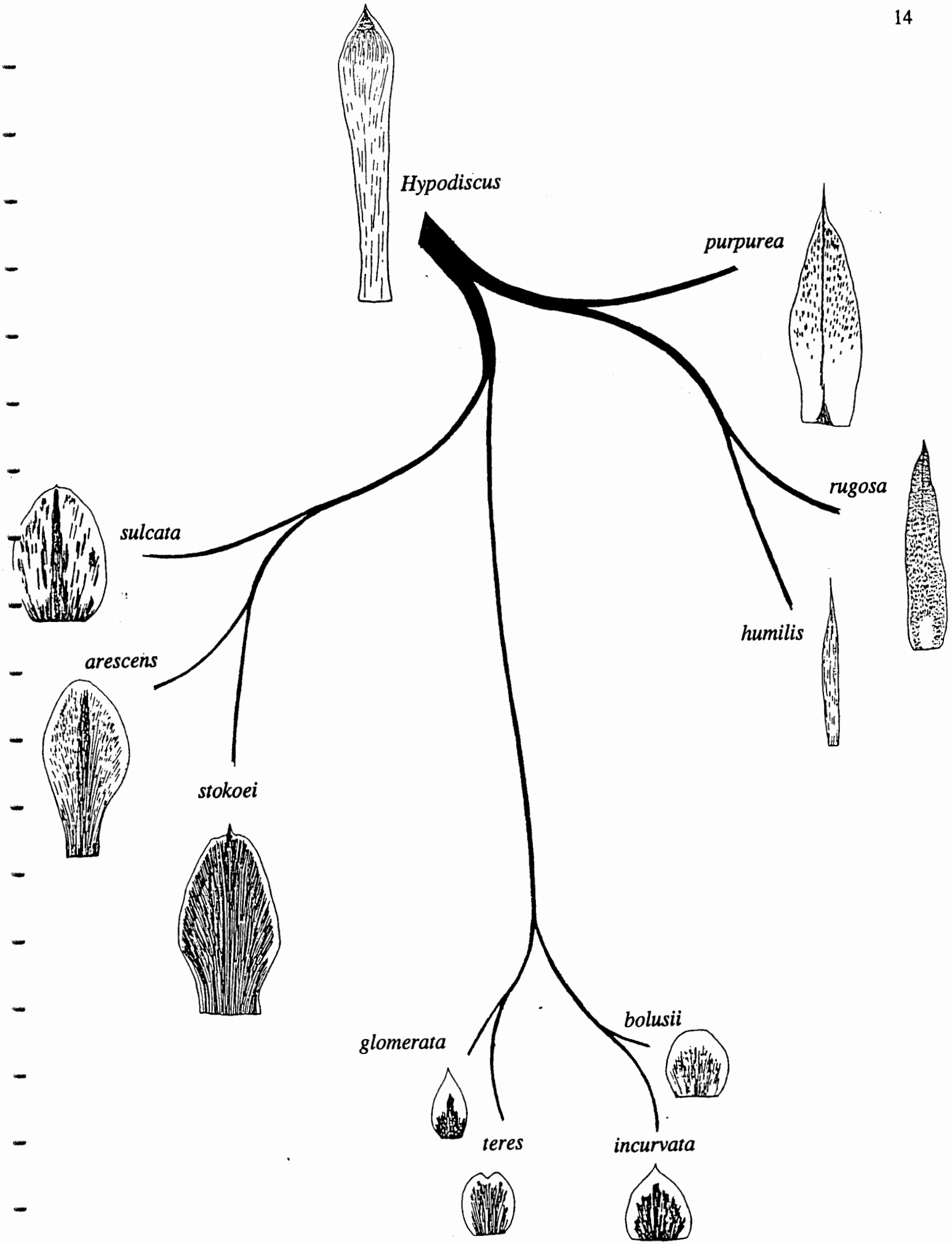


Fig. 9. Pictorial representation of inner tepal variation within *Willdenowia*.

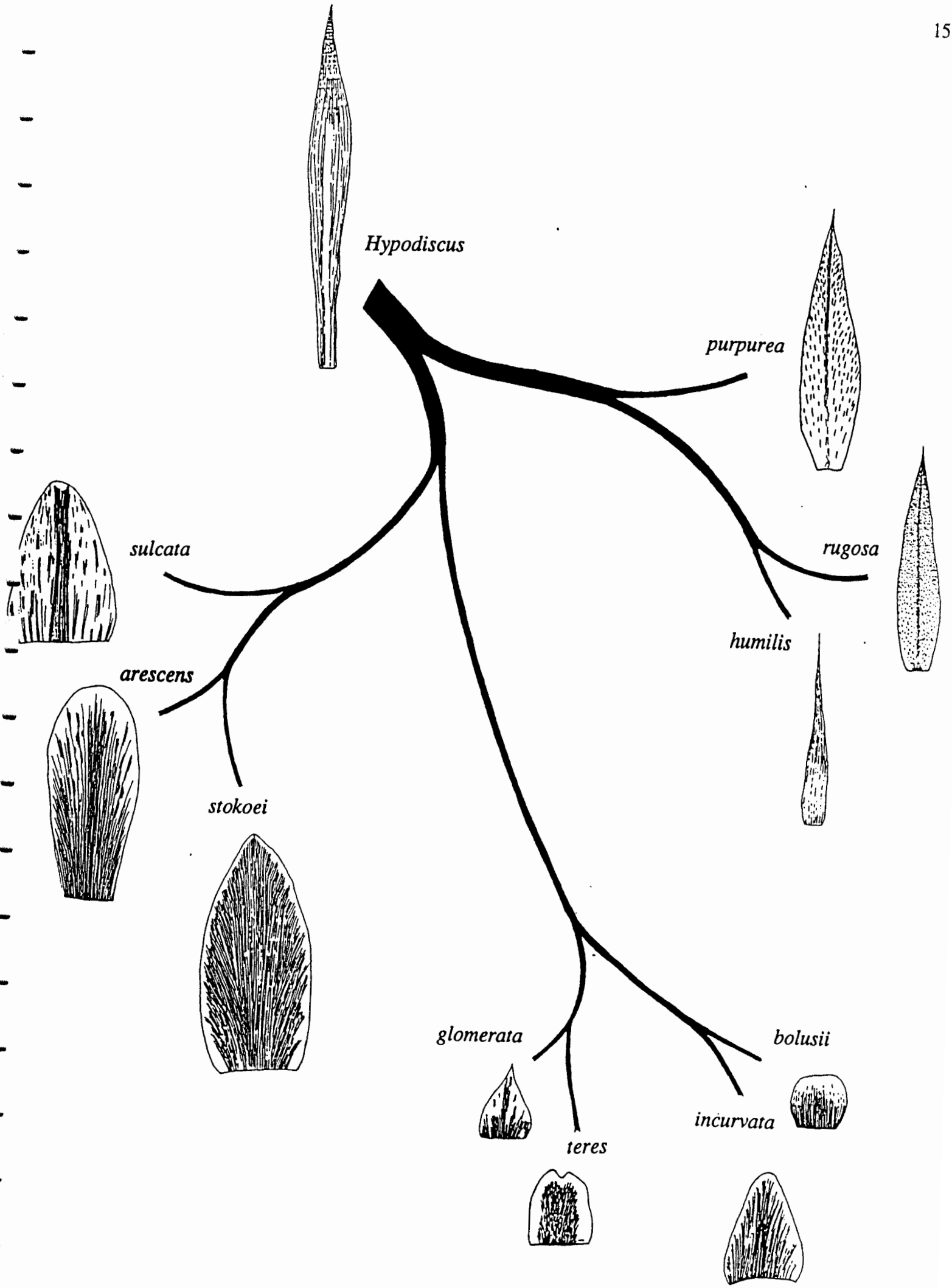


Fig. 10. Pictorial representation of outer tepal variation within *Willdenowia*.

4. DISCUSSION

4.1 Phylogeny.

When constructing a phylogeny of a particular group, unique characteristics belonging to at least two taxa of that group are of primary importance. These characteristics, termed synapomorphies, contain information relating to the evolutionary history of the study group, as they suggest a common ancestry. Unique characters belonging to a single taxa (termed autoapomorphies), do not contribute to elucidating species relationships within a particular group. *Willdenowia* possessed few suitable, informative characters which could be used to delimit clades within the genus.

The cladogram shows a phylogeny which resolved two major groups. These are:
(i) the *W.rugosa-W.humilis-W.purpurea* Pillans group and
(ii) the remainder of the taxa.

The *W.rugosa*-group is well resolved with a bootstrap value of 92%. The clade is based on characters including nut characterstics, bract number and tepal shape. Their growth form is also uniformly small (30-50cm), with a thin culm diameter. Within this group, a close relationship between *Willdenowia humilis* Nees ex Mast. and *W.rugosa* is further resolved, with a bootstap value of 68%. This species-pair is highly similar, and differs only with respect to the size of floral structures and the status of rhizomes. *W.rugosa* has an aerial rhizome, while *W.humilis* has a subterranean rhizome.

The second major group is resolved by the presence of an elaiosome, nut morphology and

tepal characteristics. Species relationships within this clade are fairly weak. This is indicated by low bootstrap values (between 7% and 45%), and the fact that all these nodes collapsed when a Nelsen consensus tree was calculated. Relationships further resolved within this second major group include the following:

(i) *Willdenowia incurvata* (Thunb.) Linder and *Willdenowia bolusii* Pillans are united by culm anatomy. Although pronounced sclerenchyma ridging has been recorded throughout the Restionaceae (Linder, 1984), this is a unique character within *Willdenowia*.

(ii) *Willdenowia teres* Thunb. and *Willdenowia glomerata* (Thunb.) Linder are resolved by a reversal in epidermal cell shape. These two species, as well as *W.incurvata* and *W.bolusii*, form a clade resolved by the markings on the innermost bract (i.e. dots), and the presence of sunken stomata.

(iii) The remainder of the species are resolved by a reversal in character 1, which is seed-coat pitting. Within this group *Willdenowia arescens* Kunth. and *W.stokoei* Pillans form a species-pair which is resolved by reversals in epidermal cell shape, and persistant styles. *Willdenowia sulcata* Mast. is the sister-group to this species-pair.

Considering the remarkable uniformity and lack of suitable synapomorphies within *Willdenowia*, this cladogram represents a suitable starting point from which to investigate speciation mechanisms in the genus. The low bootstrap values are not unusual for a cladogram of a small set of taxa, which is based on morphological data (P.Linder, pers.comm.). Confidence in species relationships resolved within *Willdenowia* should also be increased by trends in tepal shape resolved by the cladogram (see below).

4.2 Character Evolution

4.2.1 Variation of tepal morphology within *Willdenowia.*

The majority of characters used in the *Willdenowia* phylogeny, such as nut morphology and bract size and shape, are functionally highly important. Variation in these characters should therefore be constrained in an evolutionary time, providing limited opportunities for separating out monophyletic groups within the genus. Tepals, on the other hand, are of minimal functional significance with *Willdenowia* being a wind-pollinated genus. We should therefore expect a greater degree of variation in tepal morphology. This variation assumes greater importance when one considers the lack of suitable characters within *Willdenowia.* The cladogram shows a clear trend with regard to changes in tepal shape. The *W.rugosa*-clade is ancestral within *Willdenowia*, resulting in a similar tepal shape compared to the outgroup, *H.aristatus*. The trend within the *W.sulcata*-clade is to a widening of the tepal margins, with *W.arescens* and *W.stokoei* showing a narrowing at the base of the inner tepals. The final four species show a reduction in tepal length, with variations in the shape of the tepal apex. This clear trend in tepal variation shows that characters which are not constrained by functional importance (e.g. tepals in a wind-pollinated guild), can provide valuable cladistic information when investigating the phylogeny of a group.

4.2.2 Elaiosome loss within *Willdenowia.*

The *W.rugosa-W.humilis-W.purpurea* clade and *W.incurvata* are characterized by the loss of an elaiosome. This represents an interesting situation. As the presence of an elaiosome is the plesiomorphic condition for *Willdenowia*, and should represent a selective advantage (Johnson, 1992), then why would there be a reversal in this condition. In asking this question, one must assume that the conditions for analyzing the adaptive value of the eliasome have been met. These conditions include a reasonable phylogenetic hypothesis, and

a well resolved cladogram (Coddington, 1992). Other factors that would result in an inaccurate test include ambiguity of state (of character) at ancestral nodes and the failure to identify appropriate outgroups. It could be argued that the *Willdenowia* phylogeny represented here is not sufficiently resolved to allow for adaptation-hypothesis testing. The clade under question is well resolved however, and assuming the monophyly of *Willdenowia*, the ancestral condition does indeed seem to be the presence of an eliasome. Thus the conditions outlined above are largely met.

With the reduction in size of vegetative growth (including culm length and diameter) in the *W.rugosa*-clade, it is possible that the energy allocation required by the plant to produce an elaiosome of sufficient size became too great. The reward offered by an elaiosome must be sufficient for ants to transport the nut below the soil surface. As nut size (and therefore elaiosome size) decreased to a certain minimum level, below which ants could not be attracted, natural selection would have acted against the production of an elaiosome, as this would represent wasted resources. The seed coat projections that characterize *W.rugosa* and *W.humilis* could have evolved as a possible predator-deterrent, after the loss of the elaiosome had occurred.

4.3 Speciation in *Willdenowia*.

Speciation in *Willdenowia* appears to be best explained by a sympatric, as opposed to an allopatric model. This is because species-pairs are sympatric, occurring in the same general area. This would support recent studies on speciation models in the Fynbos, which invoke sympatric, ecological speciation mechanisms. (Linder and Vlok, 1991, Schutte *et al.*, in press).

4.3.1 The *W.purpurea-W.rugosa-W.humilis* clade.

W.purpurea is the sister-group to the *W.rugosa-W.humilis* species-pair. This clade is highly resolved by a distinctive growth form, seed and tepal shape. How then was *W.purpurea* isolated from the remaining members of the clade ? *W.purpurea* occurs in two isolated populations in the region of Viljoens' Pass and Franschoek Pass (fig. 1). *W.rugosa* and *W.humilis* occur south of this locality, within a band of coastal mountains (fig. 1 and 2). *W.purpurea* is thus separated in space from its sister-group at present, which suggests a possible allopatric speciation event. However, considering the proximity of the populations, co-occurrence at some stage in the past cannot be dismissed. If the populations of this clade were to have co-occurred at some stage, then another isolating mechanism would be required to separate this complex. This pattern repeats itself throughout the genus, where sister-species occur in the same general area, suggesting a form of sympatric speciation.

A possible isolating mechanism could be the different methods of wind pollination employed by the two groups. *W.humilis* and *W.rugosa* have thin, feather-like stigmas which protrude from the outer bract, which in turn is tightly pressed against the culm. The stigmas thus "hang" in the air current. *W.purpurea*, on the other hand, has a stigma which is retained within the outer bract, which acts to "scoop" the air current toward the position of the stigma. These different mechanisms could be responsible for segregating species-specific pollen grains from the air current, thus maintaining species integrity (Lotz, 1993, Niklas, 1985). The isolation effects of varying methods of wind pollination are certainly an interesting prospect for future research.

W.rugosa and *W.humilis* are a highly distinctive species pair, separated by a small number

of characters (see above).

W.humilis has a north-south distribution range, extending from Bettys' Bay in the south to Bidouwsberg in the north (fig. 1 and 2). Herbarium specimens indicate that *W.humilis* can be separated into two forms with disjunct flowering times. One form occupies lowland flats of the Cape Peninsula, with the second form found in mountainous regions. *W.rugosa* on the other hand has a highly restricted distribution range, occurring in the coastal mountains in isolated populations from Rooiels to Kleinmond. This species-pair is functionally sympatric, and thus an allopatric model of speciation cannot be invoked. An isolation of populations through phenological separation could be invoked for the *W.humilis-W.rugosa* species pair (fig. 8).

Species segregation as a result of varying phenology could play an important role in the Fynbos (P.Linder,pers.comm.). It appears that flowering time could be segregated into discrete intervals for different species in a particular area. Intervals could be as short as a few days. These intervals could also vary from region to region for the same species. For example, *H.aristatus* was observed flowering along the Perdeberg Hiking Trail, while the same species was concurrently dormant in a habitat above Silvermine, approximately 100 kms apart (pers. obs). This could be due to differences in altitude between the two sites, but it is interesting to note that another Restionaceae species was flowering at the Silvermine site - thus "preventing" *H.aristatus* from flowering at that particular time. This could be a further topic for future research. If flowering time is divided into discrete units for various species of Restionaceae within a particular area, it could help explain how so many species co-occur and yet maintain their integrity in the Fynbos biome.

This species pair could also be invoked as evidence supporting Linders' (1985a) model of speciation in the Fynbos. *W.rugosa* occurs in a narrow altitudinal range, as opposed to the wider range of *W.humilis* (fig. 8). Speciation could have been the result of altitudinally-induced ecological gradients (including temperature and rainfall), which are proposed to be necessary for sympatric speciation (Grant, 1971).

Another interesting aspect of the *W.rugosa-W.humilis* species-pair is that *W.humilis* is a sprouter while *W.rugosa* is a seeder. A noteworthy point is the increased distribution range of *W.humilis* in comparison to *W.rugosa*, a possible indication of the increased survival status of a resprouter in the fire prone vegetation of the Fynbos biome. Sprouters generally seem to have extended ranges relative to non-sprouter species (Schutte *et al.*, in press). *W.rugosa* occupies an early position in the post-fire vegetation succession. In older unburnt vegetation, no *W.rugosa* individuals were observed (pers. obs.), presumably outgrown by more robust competitors. This species is therefore dependant on fire to clear the soil surface, for new individuals to germinate and establish themselves.

A species pair can therefore partition the landscape through a change in the regeneration niche. A seeder could occupy an earlier successional position in post-fire vegetation in relation to a sprouter species, allowing a temporal segregation of the landscape.

W.rugosa has a curious mechanism relating to pollination and nut development. Young flowers are pollinated when held a short distance above the ground (± 5cm). The nut is retained on the apex of the culm, which then elongates to the normal length of the plant. The reproductive output from two seasons of flowering is therefore present on the plant at one

time. The nut is then released from a position on the apex of the plant. The function of this mechanism of elongation in the vegetative part of the plant coupled to the development of the nut is uncertain. Male flowers were observed to be aggregated at the base of female plants, which seemed to act as a "trap" for dispersed male flowers (pers. obs.). This could possibly result in enhanced pollination efficiency when flowers are closer to the ground, which would increase the reproductive output of the plant.

4.3.2 The *W.sulcata-W.arescens-W.stokoei* clade.

W.sulcata is the sister-group to the *W.arescens-W.stokoei* species pair, which are sympatric occurring in the same general region (fig. 2,3 and 5). However, the two species are separated along an altitudinal gradient (fig. 8). W.sulcata is found between 50m to 1000m, while *W.stokoei* occurs in high-altitude habitats, in the range from 1900m to 2100m above sea level. The anatomy of *W.stokoei* is unique within the genus *Willdenowia*, having two distant bands of cells in the chlorenchyma layer. The outer layer consists entirely of false pillar cells, while the inner layer consists of chlorenchyma cells. It has a stout, relatively short growth form with a thickened sclerenchyma layer. All these unique autapomorphies are possible adaptations to surviving at high altitude, making it a member of the recently described Cape alpine flora, a distinct set of high altitude species (Linder et.al.,1993).

It is apparent that *W.arescens* and *W.stokoei* are isolated through ecological constraints, resulting from the steep ecological gradients which are present within the Fynbos biome (see above). The selective pressures of occupying an alpine habitat could have resulted in *W.stokoei* evolving specialized structures to tolerate extreme environmental conditions. The disjunction in environmentally induced selective pressures between the respective habitats of

this species-pair, which is a function of altitude, has possibly resulted in a speciation event. This is the same mechanism proposed to be driving speciation in the *Restionaceae* genus *Rhodocoma* (Linder and Vlok, 1991).

The phenology of *W.sulcata*, the sister-group to the above species-pair, is uncertain. There is a possibility that speciation occurred as a result of isolation through flowering time (fig. 8). A genetic barrier between populations would develop as flowering time became asynchronous.

4.3.3 The *W.bolusii-W.incurvata* species pair.

This species pair is characterized by unique synapomorphies within *Willdenowia*, including sclerenchyma rays which penetrate through to the epidermis, an increased number of silica bodies and a mid-winter phenology. This species pair is geographically separated, with no overlap in distribution ranges (fig. 3 and 5). Allopatry could be invoked to explain speciation in this species pair, which is separated in space. This is the only occassion where a species pair is clearly geographically separated in *Willdenowia*. Again, this species-pair occupies different altitudinal bands (fig. 8), with *W.bolusii* restricted to higher altitudes.

W.incurvata lacks an elaiosome and has a unique stylar form within *Willdenowia*. The style consists of two flattened, cushion-shaped structures which remain attached to the nut, possibly indefinitely. Nuts which had already been released from the flower head still had the style attached (pers. obs.). Bearing this in mind, the stylar material could possibly function as an elaiosome. *W.incurvata* appears to be partly serotinous, with mature seeds being held on the plant (pers. obs).

4.3.4 The *W.glomerata-W.teres* species pair.

This species pair is resolved on the basis of interrupted parenchyma. Their distribution range is partly sympatric, co-occuring in the west. This would suggest a sympatric speciation event. Segregation in the landscape could have resulted from a difference in regeneration status, with *W.glomerata* a seeder and *W.teres* a sprouter.

4.4 The sprouter/seeder pattern in the Willdenowia phylogeny.

Evidence as to whether sprouting after fire is a derived or ancestral condition is contradictory (le Maitre and Midgley, 1992, Kornas, 1978). Differential selection pressures particular to a certain region or plant group, have resulted in no consistent trend regarding ancestral regeneration status.

The plesiomorphic condition for Willdenowia is the ability to resprout after fire. Three species have lost the ability to resprout after fire, including *W.rugosa, W.incurvata* and *W.glomerata* (fig. 8). By optimizing down to the nodes, it is apparent that re-seeder species have evolved from sprouter species (i.e. sprouting is the plesiomorphic condition). The question is why this should occur and what the implications of re-seeding being a derived trait are.

In a flora such as the Fynbos, where fire is the dominant disturbance factor, the ability to resprout after fire is of great ecological and evolutionary significance (le Maitre and Midgley, 1992). By resprouting after fire, a plant is able to persist in the landscape for an indefinite period in evolutionary time. In contrast, a seeder species is more vulnerable to local extinction as a result of stochastic fire intervals, which are characteristic of the Fynbos (le

Maitre and Midgley, 1992).

If a population can lose the ability to resprout after fire (for which there should be no selective pressure in a fire-prone environment such as the Fynbos), then there should be an equal chance for a population to re-gain the ability to resprout after fire (for which there should be strong selective pressure). As the latter does not occur within the Willdenowia phylogeny, the answer would seem to lie in the differential persistence ability of taxa with opposite regeneration strategies.

If the rate of random evolutionary mutations leading to a switch in survival strategy in either direction is equal, then there must be another factor, other than selective pressure which is responsible for the pattern observed. Possibly, a re-seeding species does not persist for long enough in evolutionary time to give rise to new lineages. A sprouter, on the other hand, which persists indefinitely in the environment could give rise to a greater number of new lineages, some of which may lose the ability to resprout after fire (as a result of random mutations).

It could thus be a question of survival time, a re-seeder not persisting for long enough for random evolutionary events to occur within a lineage. The above pattern and its inferred process, if correct, has important implications for the long-term conservation of species and evolutionary processes. According to the above model, sprouter species are of greater conservation value than their re-seeding counterparts, with regard to their ability to give rise to new lineages. Extinction of a sprouter could lead, indirectly, to an erosion of biodiversity through a loss of evolutionary potential to generate new species. A re-seeder, in comparison,

has relatively less potential to increase biodiversity over evolutionary time, through speciation events.

Therefore, the hypothesis is that the ancestral regeneration status in the *Restionaceae* is the ability to resprout after fire, and that this process has had a considerable impact on speciation within the family.

This study should not be regarded as conclusive evidence for the pattern described above. Rather, it should be seen as a starting point against which future research can be compared. Future study requires phylogeny construction of other genera within the *Restionaceae*, and an investigation of these evolutionary histories with regard to the pattern and distribution of seeders and sprouters. An ideal study group should include a greater proportion of re-seeders than Willdenowia, to assess the consistency of this pattern. It should also be possible to resolve a robust cladogram of the study group, to obtain the most accurate phylogeny (and thus species groups) possible. This will provide an accurate representation of which taxa (and therefore characters) have given rise to others.

Future research should also concentrate on the seeder-sprouter dichotomy in other Fynbos families such as the *Proteaceae, Rutaceae* and the *Ericaceae*. What is the pattern in the Ericaceae and in the largest Fynbos legume genus *Aspalathus*, in which most species are re-seeders (le Maitre and Midgley, 1992). In these groups it is thought that susceptibility to local extinction as a re-seeder, as a result of stochastic declines in population number are due to fire disturbances, increases potential for speciation (Cowling *et al.*, 1992). This could be due strong selection, resulting from an increase in generation turnover or the stochastic

decline of seeder populations to low numbers. In contrast, sprouter species which are persistent, are in turn resistant to speciation (Cowling *et al.*, 1992). By investigating these groups, it will be possible to assess whether or not a re-seeder can, in fact, give rise to other re-seeders and with what repeatability this occurs. It is possible that the evolution of regeneration strategy has varied between famalies within the Fynbos (le Maitre and Midgley, 1992). Evindence for this includes varying proportions of seeders and sprouters in different famalies, which is a possible indication of different processes within these famalies. Also, the organs used to inititiate sprouting after fire differ markedly between famalies (e.g. lignotubers in *Proteaceae* vs. dormant shoot buds in the *Restionaceae*).

5. CONCLUSION

5.1 Speciation in the Fynbos.

Although ecological gradients are the dominant force behind speciation in *Willdenowia*, other factors such as differing phenology, pollination syndromes and geographical separation could also be invoked as playing a role. This suggests that there is no single unique factor at work in the Fynbos that has resulted in the increased diversity of the region. Rather, a number of forces appear to have acted in concert, to produce the pattern that we see today. Thus I would suggest that any attempt to isolate a single factor as causative to the increased diversity of the Cape Flora would be unfruitful.

5.2 Model of seeder vs. sprouter speciation potential in the Fynbos.

In a period where the fire-regime favours the establishment of re-seeders (i.e. 10-30 year intervals), then this could result in a rapid burst of speciation events, as a result of the factors outlined above (i.e. increased generation turnover and extinction probability). This could have been the scenario for genera such as *Aspalathus*, although this would have to be resolved through phylogenetic analysis. However, as soon as the fire regime moves out of this favourable "window" for re-seeders, then sprouters become the dominant group producing speciation events. This would follow the pattern described above, where re-seeders suffer repeated local extinction and are not present in the landscape for a sufficient period to result in speciation events.

APPENDIX 1

TABLE 1. Data set and characters used in the cladistic analysis.
[x] = inapplicable.

Hypodiscus	00000	00112	11001	01001	11000	10100	0
W. arescens	11011	00111	01001	11311	11000	02100	0
W. bolusii	01000	00111	01000	00111	10111	10110	1
W. glomerata	01011	00111	11000	00311	10000	01101	1
W. humilis	00130	110x0	10001	01011	01000	03101	0
W. incurvata	1101x	000x1	0x110	00211	00111	11110	0
W. purpurea	00131	000x0	10011	01010	21010	11102	0
W. rugosa	00130	110x0	10001	01001	11010	03100	0
W. stokoei	11011	000x1	00100	01311	11000	03202	1
W. sulcata	11010	00101	01110	01211	01001	10000	0
W. teres	00000	00111	01000	00110	20000	00100	0

Character List

Seed Morphology [fig. 11 (a) to (d)].

1. seedcoat: smooth (0) or pitted (1)
2. Seedbase pitting: absent (0) or present (1).
3. Shape: round (0) or long thin (1).
4. Apex: concave (0) or flat convex (1);[additive].
5. Apex pointed remains: absent (0) or present (1).
6. Seedcoat projections: absent (0) or present (1). Pillar-like projections cover the seed, and project from the apex of the seed. The shape of the *W.purpurea* nut is the same as the two species above, but lacks the conical projections.
7. Conical projections on apex: absent (0) or present (1).
8. Elaiosome: absent (0) or present (1).
9. Elaiosome: solid (0) or ribbed (1). The solid elaiosome of *W. arescens* appears to have a different origin from the remaining species which possess a ribbed elaiosome, which appears to be derived from the tepal bases.

Bract and Floral Morphology

10. Number of bracts: 0-5 (0), 6-15 (1) or >15 (2)[additive]. The trend within *Willdenowia* is toward a reduction in the number of bracts, relative to the outgroup, which has >30 bracts. The size, shape and number of bracts is constrained in evolutionary terms, as a result of their vital importance in wind pollination. Variation is also dependant on the type of wind-pollination mechanism employed (e.g. wind-tripping vs. wind-scooping.
11. Outer tepal: obtuse or rounded (0); acute or acuminate (1).
12. Spathe/First bract different: present (0) or absent (1).
13. Inner/Outer tepals different: no (0) or yes (1).

Tepal shape. (fig. 9 and 10).

The *W.rugosa*-group shares a distinctive outer tepal shape, which is similar to that of

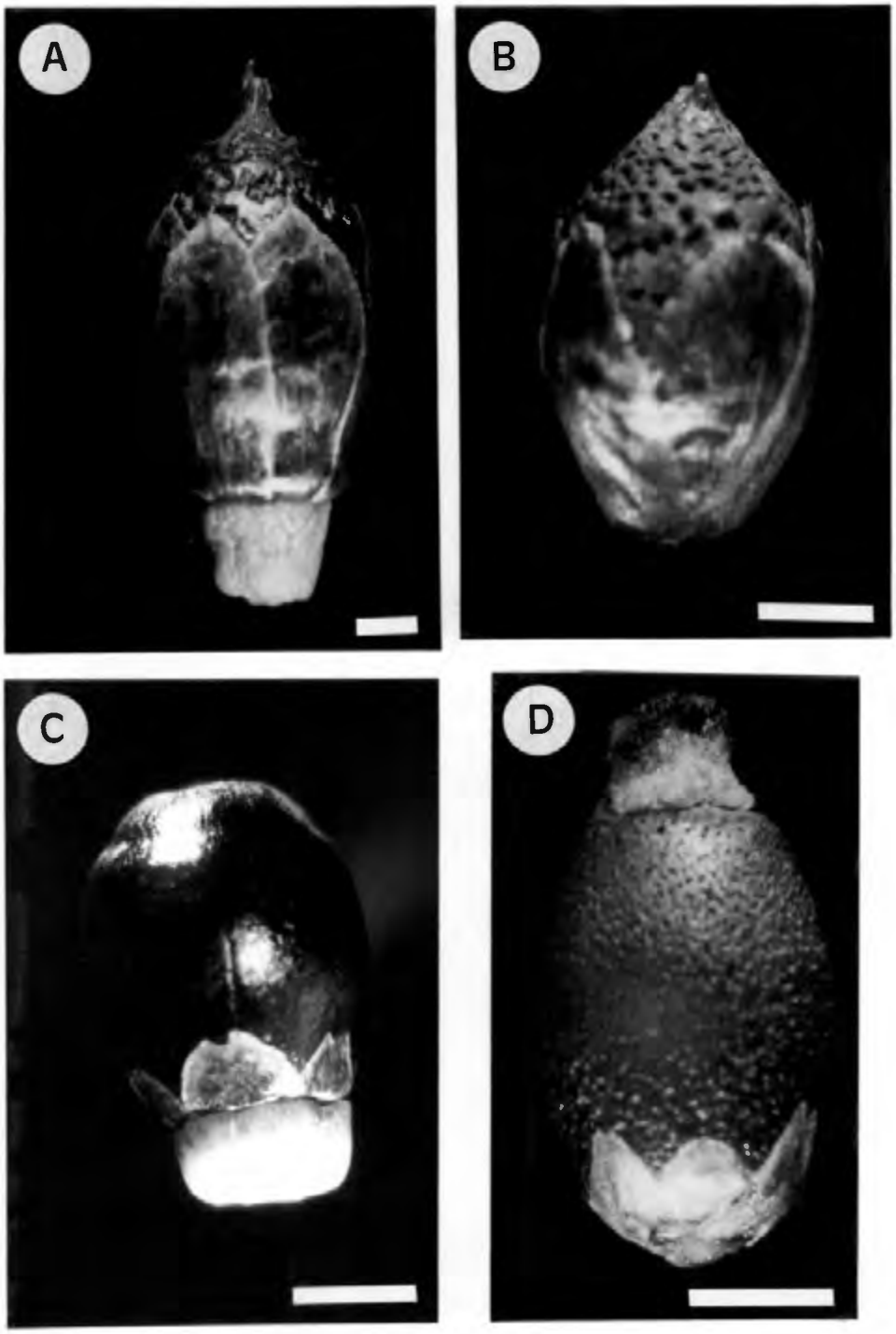


Fig. 11 (A) - (D). Nut morphology of various species of *Willdenowia*. **A**-*W.arescens* (ESTERHUYSEN 30713) showing seed-coat pitting and eliaosome. **B**-*W.stokoei* (ESTERHUYSEN 28484) showing seed coat pitting. **C**-*W.teres* (PILLANS 23143). **D**-*W.incurvata* (LEVYNS 11682) showing style form. Scale bar: 2mm.

H.aristatus. Tepal shape is an important character for delimiting groups within *Willdenowia*, and three possible groupings can be recognized from the shape of the inner tepals.

14. Darkened midrib: absent (0) or present (1).
15. Outertepal base: broad (0) or narrow (1).
16. Outertepal shape: ovate or lorate (0); spathulate (1).
17. Outertepal: short (0) or long (1).
18. Outer tepal shape: tapering (0); round (1) or flat at base (2) [additive]
19. Innermost bract: Dots (0); Lines (1).
20. IMB: white (0) or coloured (1).
21. IMB shape: long/thin (0); square (1); round (2)[nonadditive].
22. Innertepals colouring from base out: present (0) or absent (1).

Culm Anatomy [fig. 12 (a) to (e)].

The overall culm anatomy of *Willdenowia* (as in the Restionaceae) is remarkably uniform (Cutler, 1969), consisting of (i) and epidermal layer (1 cell thick), (ii) a photosynthetic chlorenchyma layer (2-3 cells thick), (iii) a parenchyma layer (1-2 cells thick), (iv) a sclerenchyma layer and (v) central ground tissue. The centre of the culm is also occasionally hollow. Observed anatomical characteristics of *Willdenowia* were verified using Cutler (1969) and van Greuning and van der Schijff (1974).

23. Chlorenchyma: whole (0) or blocks (1). Chlorenchyma is sub-divided into blocks by the sclerenchyma sheath, which penetrates through the chlorenchyma to the epidermis at regular intervals. This produces the characteristic vertical striations on the culm surface of *W.bolusii* and *W.incurvata*.
24. Stomata: on surface (0) or sunken (1).
25. False pillar cells: absent (0) or present (1). van Greuning and van der Schijff (1974) refer to these cells as support cells. They are thought to serve as a buffer against radial pressure (Gilg, 1891, as cited by van Greuning and van der Schijff, 1974).
26. Parenchyma: continuous (0); interrupted (1). van Greuning and van der Schijff (1974) reported interrupted parenchyma in both *W.teres* and *W.glomerata*. However, two herbarium specimens of each of these species provided no evidence to support this observation. This suggests that interrupted parenchyma is a variable trait at the intraspecific level, and should thus be used with caution as a characteristic to group taxa within *Willdenowia*.
27. Tannins: 50-100 (0); 30-50 (1); 10-30 (2) 0-10 (3) [additive]
28. Shape Epidermal cell: square (0) or rectangular (1) or elongated (2) [additive].
29. Number of cells in sclerenchyma: <10 (0) or >10 (1).
30. Number silica bodies: 1-2 (0); 3 (1); 4-5 (2) [additive].Silica bodies are found aggregated between the sclerenchyma ridges below the parenchyma layer. Cutler (1969), records *W.glomerata* as having no silica bodies, which is incorrect.
31. Thickening Epidermal cells: absent (0) or present (1).

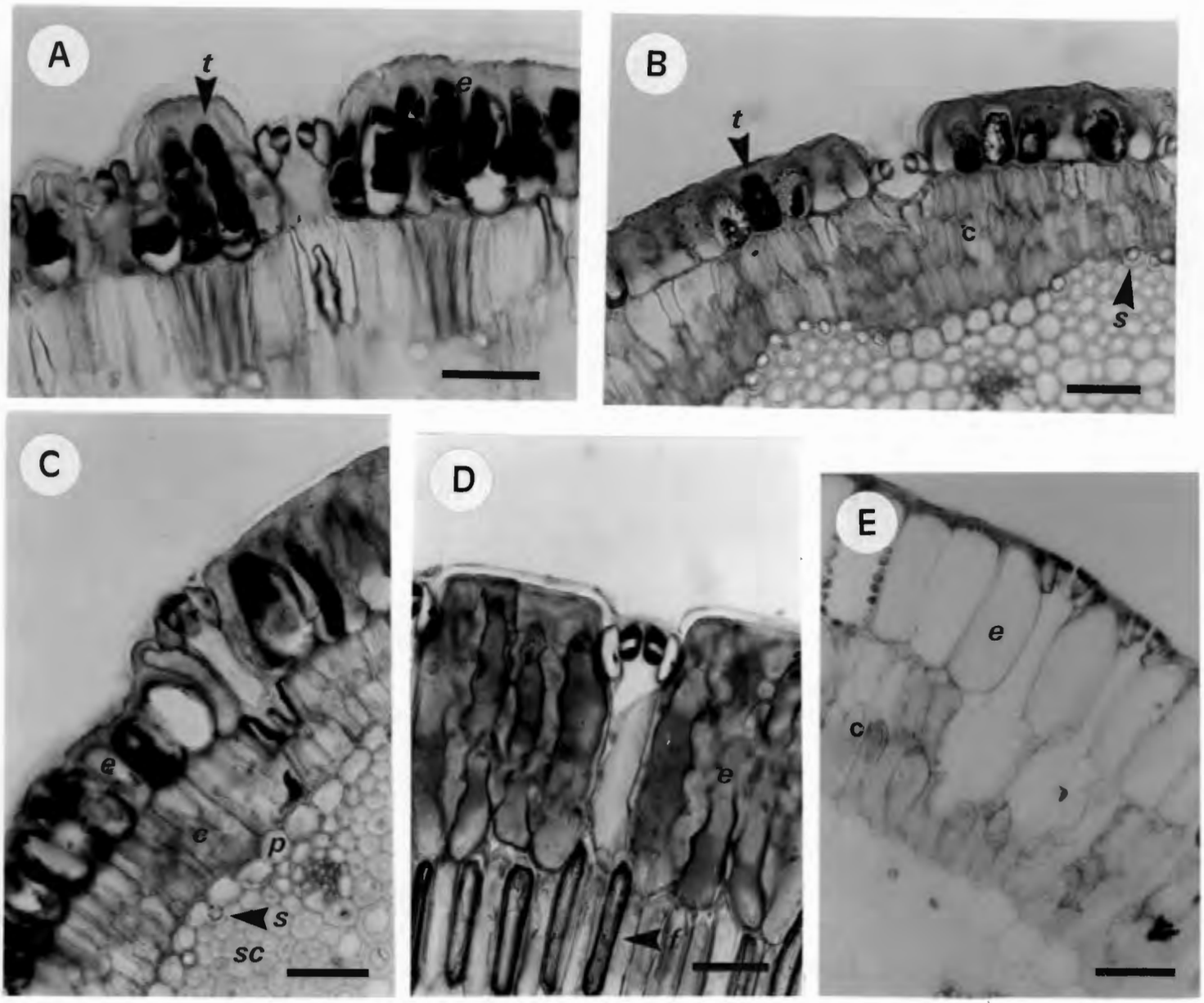


Fig. 12 (A) - (E). Culm anatomy of various species of *Willdenowia*. **A**-*W.bolusii* (LEVYNS 10746). **B**-*W.purpurea* (ESTERHUYSEN 33062). **C**-*W.arescens* (ESTERHUYSEN 30713). **D**-*W.stokoei* (ESTERHUYSEN 30474). **E**-*W.humilis* (ESTERHUYSEN 35216). *e* Epidermis, *c* chlorenchyma, *f* false pillar cell, *p* parenchyma, *sc* sclerenchyma, *s* silica body, *t* tannin cell. Scale bar: 50 μm.

APPENDIX 2

Vouchers for the anatomical and seed morphological studies of the *Willdenowia* species.

Species	Voucher	Reproductive	Culm	Nuts
H.aristatus	31607 Ee			+
	6772 Ee	+	+	
W.arescens	30713 Ee		+	+
	Esterhuysen S.N. September 1941	+		
W.bolusii	10746 MrL		+	+
	35201 Ee	+		+
W.glomerata	30743 Ee		+	+
	24960 GvN	+		
	8683 Pillans		+	
W.humilis	35216 Ee		+	+
	35354 Ee	+		
W.incurvata	11682 MrL	+	+	+
	13222 HB	+		
W.purpurea	33062 EE		+	+
	33371 EE	+		
W.rugosa	35890 EE	+	+	+
W.stokoei	28484 EE			+
	30474 EE	+	+	
W.sulcata	30589 EE		+	+
	Stokoei S.N.	+		+

	24485 Acocks				+
W.teres	23143 NsP	+		+	
	30787 EE			+	
	2344 Fourcade				+

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